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THE FATE OF STREPTOCOCCUS HEMOLYTICUS IN THE GASTRO-INTESTINAL CANAL

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Dangerous streptococci, especially Str. hemolyticus, are found very commonly in the throat. Our observations[1] show that in the crypts of the faucial tonsils hemolytic streptococci occur in nearly 100%. Often they occur in large numbers on the pharyngeal mucosa in normal persons, and especially in persons suffering with various respiratory infections, in which these organisms so often play an important rôle. Large numbers of streptococci are therefore constantly passing down the esophagus into the stomach in both normal and diseased persons. Furthermore, during milk-borne epidemics of streptococcus sore throat, milk from infected cows containing large numbers of streptococci is swallowed. The fate of these virulent hemolytic streptococci in the gastro-intestinal canal is the subject of this paper.

Three questions arise: First, the possibility of secondarily infecting the intestinal canal; second, the invasion of the body through the wall and third the possible danger of transmission of dangerous streptococci through sewage, etc.

As is well known, the gastric contents exercise a strong bactericidal effect on bacteria entering the stomach. Certain acidophils are able to live and develop in the stomach; spores and certain fungi, on account of their resisting properties, can do likewise. But the great mass of bacteria that enter the stomach through the act of swallowing are killed, and therefore the food passing into the duodenum contains relatively few organisms.

While these statements are generally true, there are exceptions due to several reasons; bacteria become enmeshed in particles of food and are thereby protected from acid until they enter the duodenum. This is especially true in cases of hypermotility. In achylia the bacteria pass through the stomach little affected by the feebly acid gastric contents.

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[1] Pilot and Davis: Jour. Infect. Dis., 1919, 24, p. 386.

Consequently, under certain conditions many bacteria from the throat may pass through the stomach unharmed and invade the intestinal canal.

Little work has been done to determine specifically the effect of the intestinal juices on bacteria, and apparently nothing has been done to determine their effect on virulent streptococci.

The rabbit is especially susceptible to experimental infections with Str. hemolyticus; for this reason it was selected for certain experiments designed to test the action of the gastro-intestinal contents on this organism.

In the examination of material for hemolytic streptococci the following routine method was employed: Plain blood agar plates were made, using 0.5 c c of human blood to 5 c c of medium. This medium was inoculated usually with a small quantity of intestinal contents, which, whenever necessary, was properly macerated in sterile fluid. Several plates were made in order to obtain proper dilutions. The hemolyzing colonies were isolated and, when necessary, confirmatory tests were made. By hemolytic streptococci I mean streptococci having a wide, clear zone of complete hemolysis (type B or Theobald Smith) and not the imperfect hemolyzers (type A).

The intestinal contents from three normal rabbits living on a routine diet of carrots, hay and water were examined for hemolytic streptococci in the following manner: The animals were killed, the abdomen at once carefully opened, and several specimens of the contents were obtained from stomach, duodenum, ileum and colon, and plated on blood agar. In none of the specimens were hemolytic streptococci isolated; green streptococci of the fecalis type were common. Numerous other organisms, including hemolytic colon bacilli, appeared on the plates. Another normal rabbit was placed under observation for a period of nearly 3 weeks and each day one or more specimens of feces were cultured. Nineteen specimens thus collected were examined and all yielded negative results as to hemolytic streptococci. Many green producing streptococci appeared. This preliminary experiment was made primarily to determine the incidence of hemolytic streptococci in the intestinal canal of normal rabbits.

The following experiment was carried out to determine whether or not hemolytic streptococci fed to rabbits would live in the gastro-intestinal canal and cause symptoms: It was found that feeding was unsatisfactory and the streptococci were therefore introduced by means of a small catheter which serves as a satisfactory stomach tube for this animal.

Three healthy rabbits were carefully selected and their stools examined for hemolytic streptococci, with negative results. They were then given, by means of the rubber catheter, suspensions of streptococci. Three strains were given, one an old laboratory strain that appeared to be somewhat hardy and had lost most of its virulence (3 c c of a broth culture intravenously did not cause lesions) and two virulent strains recently isolated, one from a tonsillar abscess which killed a rabbit in doses of 1 c c given intravenously, and another isolated from the spinal fluid of a case of meningitis. Each day for ten days, from 10 to 20 c c of a 24-hour milk and broth culture of the relatively avirulent strain were given to the three rabbits, and each day the feces were collected and examined. In rabbit 1 on the ninth day a very considerable number of streptococcus colonies appeared on the plates which, on examination, were shown to be identical with those injected into the stomach. These cocci appeared suddenly, not being noted before in the feces. On the following day also a smaller number of the same streptococci were found, but on the third day none appeared. At the end of fourteen days, and for six days thereafter, the strain of streptococci from the tonsillar abscess was fed to the rabbit. No hemolytic streptococci were found in the feces in this time. Then for the next twelve days the freshly isolated strain of hemolytic streptococci from a fatal case of meningitis was given by mouth. During this time no hemolytic streptococci appeared in the feces. At no time did this rabbit show any evidence of infection or appear abnormal in any way. It was noted that on the two days when the hemolytic streptococci were found in the feces the latter were slightly more moist and softer than usual. Otherwise they remained throughout normally firm and well formed.

In rabbit 2 the streptococci were given as in 1. In this animal, on the fifth day after giving the meningitis streptococcus, there appeared in the feces numerous typical hemolytic streptococci like those given by stomach. They appeared suddenly, and the feces were slightly moist. On the following day they disappeared and were not recovered thereafter.

In rabbit 3 on the sixth day after feeding the avirulent streptococcus, streptococci appeared suddenly in the stools in large numbers. Two days later they were not found. Again six days after beginning the administration of the meningitis streptococcus about 25 colonies appeared on the plates, which proved to be identical with the original meningitis streptococcus. They did not reappear.

At the end of 31 days, during which these observations were being conducted, the three animals were killed and specimens for examination were obtained from various levels of the gastro-intestinal tract: 2 from the stomach, 8 from the small intestine and 4 from the cecum and large intestine. Cultures made from this material in the 3 rabbits in every case yielded no hemolytic streptococci. The stomach and intestinal mucosa appeared normal, as did all the other organs in the animals.

It appears from these experiments that when large numbers of streptococci are introduced they are usually killed promptly, presumably in the stomach, but that occasionally, as in each of the three animals, they may appear for a short interval in considerable numbers in the feces. They do not, however, permanently seed the intestinal canal with streptococci as evidenced by their absence in the examination made at necropsy. Nor is it possible, judging from these experiments, in this way to infect the rabbits or cause lesions of the intestinal tract.

In the light of these experiments it is difficult for me to understand the results of certain workers who report finding intestinal lesions and generalized infections followed by death after the administration by mouth of even small quantities of streptococci. Bail[2] states that he was able to so infect rabbits from 10 to 12 weeks old by injecting into the empty stomach far smaller quantities of streptococci than were used in my experiments. He believes that virulent pyogenic organisms may pass through the uninjured intestinal mucosa and cause a general infection. Holst[3] reports similar results with animals and with himself after feeding with cultures of streptococci. These results are not in accord with my own, nor are they in harmony with the general fact that in streptococcus pneumonia and other respiratory infections large numbers of streptococci are swallowed, but are not found, at least in large numbers, in the feces. The organisms may not have been hemolytic streptococci.

It was thought that perhaps the intestinal canal might become infected during a generalized infection with streptococci. Three rabbits were therefore inoculated with a virulent hemolytic streptococcus intravenously. Each developed severe multiple arthritis and had fever, loss of weight and other evidence of a generalized infection. Twelve days later the three animals were killed and cultures made from stomach, duodenum, ileum and cecum, as well as from heart blood and joint fluid. The various involved joints yielded streptococci in large numbers as did also the heart blood in one rabbit; but no hemolytic streptococci were found in the intestinal contents. In the duodenum very few bacteria of any kind were found in the intestinal contents; lower down the numbers were greatly increased. It appears therefore, that though the streptococci are in the blood and other tissues, the intestinal canal may remain free from these organisms.

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[2] Arch. f. klin. Chir., 1900, 62, p. 369.

[3] Baumgartens Jahresbericht, 1895, 11, p. 52.

HEMOLYTIC STREPTOCOCCI IN HUMAN FECES

There is little in the literature concerning the occurrence of hemolytic streptococci in human feces. Broadhurst[4] isolated strains from human feces, dog intestines and stomach, and various other sources, but does not clearly state whether they are the wide hemolyzers of the beta type.

A routine examination of 53 specimens of human feces was made. They were obtained from a variety of persons many of whom were patients in a large hospital and suffering with a variety of diseases, few of which were intestinal, however. Since we have shown that practically all tonsils contain hemolytic streptococci,[1] and since a large proportion of persons harbor many of these organisms at all times in their throats, we may be sure that many, if not all, of these patients were swallowing large numbers of them constantly in their foods. The feces were collected and cultured usually within a few hours after evacuation. Small particles were fished and stirred in a small quantity of broth or salt solution from which several blood-agar plate cultures were made. Suspicious colonies were fished and subjected to proper tests. In none of the 53 specimens were hemolytic streptococci demonstrated. In a number of the specimens broth was inoculated with a small particle of feces and incubated for from 12 to 18 hours, and from this material plates were made. These examinations also failed to reveal this organism in the specimens examined.

Since scarlet fever patients practically always have hemolytic streptococci in their throats and usually in very large numbers,[5] the feces from four cases were examined to determine if in this disease these cocci passed through the canal. Examinations made in the usual way failed to reveal the hemolytic variety; many green streptococci were always present. Small particles of three of the specimens were suspended in salt solution and inoculated into the peritoneal cavity of three white mice. One white mouse was sick for two days, but recovered. On the fourth day examination revealed no streptococci in the peritoneal cavity. A second mouse died two days later. Small green streptococci were in the heart blood, but no hemolytic streptococci were found in the blood or peritoneal cavity. The third mouse died after four days. A few B. coli were in the peritoneal cavity and the heart blood was sterile; no hemolytic streptococci.

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[4] Jour. Infect. Dis., 1915, 17, p. 277.

[5] Ruediger: Jour. Infect. Dis., 1906, 3, p. 755.

Thus, in none of the feces from normal or from the scarlet fever patients examined were hemolytic streptococci found. In this connection Kraft[6] working in this laboratory has examined 125 normal and pathologic appendices, cultivating the contents and also the scrapings of the mucosa. In 48 normal appendices he found only 2 in which hemolyzing streptococci occurred, and in these there were only a few. In 77 pathologic appendices examined he found 4 containing this organism and in decidedly larger numbers than in the normal ones.

The following experiment was made to determine how long hemolytic streptococci will live in feces: A specimen of fresh human feces was examined and no hemolytic streptococci found. A virulent freshly isolated throat hemolytic streptococcus was mixed with the feces, placed in the icebox, and at daily intervals cultures were made. At the end of seven days under these conditions the hemolytic streptococci seemed as numerous as in the first control. In a similar way, feces were mixed with hemolytic streptococci and kept at incubator temperature and cultivated from time to time. The results were different. While at the end of two hours numerous hemolytic streptococci were present, at the end of 24, 48 and 72 hours none were obtained in the feces. In the incubator the streptococci appear to be rapidly overgrown and destroyed by the normal fecal organisms. It should be pointed out that conditions in the test tube are not directly comparable to those in the intestinal canal where fresh alkaline intestinal secretions are being constantly poured, the reaction being thereby maintained normally not far from the neutral point. In the intestine also the mucosal surface might under certain conditions, especially pathologic, favor the development of hemolytic streptococci.

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[6] Unpublished observations.

ACTION OF GASTRIC JUICE ON HEMOLYTIC STREPTOCOCCI

Strauss and Wurtz[7] showed that the destructive effect of stomach contents on bacteria, in vitro, runs parallel with the HCl content. Others have obtained evidence that in addition to the acid effect the pepsin may exert some germicidal action. This is probably slight, if it exists at all. The concentration of HCl in gastric contents varies according to Clark and Lubs,[8] from P_H 0.9 to P_H 1.6. This is far below the limiting H-ion concentration (5.2) at which hemolytic streptococci will develop, as determined by Jones[9] and others.

With concentrations of HCl in the stomach in ranges approaching normal we should, therefore, expect the hemolytic streptococci to be rapidly destroyed and the contents to become free from these organisms in a short time. This does occur.

In an examination of 15 stomach contents no hemolytic streptococci were found. In these the total acidity ranged from 14 to 85. In four no free acid was present when tested with the dimethyl test. In these cases of low acidity certain varieties of bacteria were far more numerous than in the other specimens, but hemolytic streptococci were not found.

In order to test the direct bactericidal action of the gastric juice on hemolytic streptococci pure suspensions of this organism were added to 5 c c of the stomach contents. Of 15 samples thus tested, 11 had free acid with total acidities ranging from 28 to 85, and in all the hemolytic streptococci were dead in five minutes, many of them in two minutes. Of the four samples with no free acid and with total acidities of from 14 to 19, the hemolytic streptococci lived from one to three hours, but were dead in twenty-four hours. Presumably in the stomach the action would go on somewhat more effectively than in the test tube owing to more favorable conditions. These results strikingly illustrate the effectiveness of proper acidity in the stomach in determining the character of the gastric flora.

In the older literature there are many references to work on streptococcal enteritis which seems to center largely around that of Escherich[10] and his school. The examinations made at that time do not permit one to classify the streptococci accurately, but there are reasons to believe that many, if not all, belong to the streptococcus fecalis group. Baermann and Eckersdorf[11] describe a series of cases of croupous enteritis simulating dysentery in which they say streptococci were found and cultured in large numbers from the stools and the intestinal exudate, and were strongly hemolytic on blood plates. Holst[12] describes a family epidemic of enteritis arising from the use of milk from a sick cow. These observations, together with several others of a similar character, suggest that streptococci of the hemolytic variety may at times play a rôle in gastro-intestinal infections, even as primary invaders. However, much of the literature is old and consequently the data are difficult to analyze in the light of more modern methods and conceptions. The problems involved, therefore, in the possible relation of hemolyzing streptococci to pathologic lesions (enteritis, diarrheas, dysenteries, etc.) in the intestinal canal require reinvestigation, and certain work along these lines has already been undertaken and will be reported shortly.

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[7] Quoted from Kolle and Wassermann, 1914, 1, p. 993.

[8] Jour. Bacteriol., 1917, 2, p. 218.

[9] Jour. Infect. Dis., 1919, 24, p. 386.

[10] Die Darmbacterien des Säuglings, 1886.

SUMMARY

Hemolytic streptococci do not occur normally in any appreciable numbers in the gastro-intestinal canal of rabbits. When introduced into the stomach of rabbits in large numbers, virulent hemolytic streptococci may occasionally pass through the canal and appear in the feces. Examination of the gastro-intestinal canal at various levels in rabbits thus fed indicates that the hemolytic streptococci do not develop appreciably in the intestines, nor do they readily gain a permanent foothold there. Rabbits with generalized streptococcus infection in joints, blood, etc., showed no hemolytic streptococci in the intestinal contents.

Gastric juice of normal acidity from man and from rabbits kills hemolytic streptococci in from two to five minutes. Gastric juice in achylia may not kill them in several hours.

In normal human feces hemolytic streptococci were not found in 53 cases.

Hemolytic streptococci when mixed with normal human feces will live in the icebox for at least several days. In the incubator they tend to die out rapidly.

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[11] München. med. Wchnschr., 1909, 56, p. 1169.

[12] Baumgartens Jahresbericht, 1895, 11, p. 52.